AMENDMENTS TO THE SPECIFICATION

In the Specification

Please substitute the following amended paragraphs (deleted matter is shown by strikethrough for six or more characters and double brackets for five or less characters and added matter is shown by underlining):

Page 1, before line 4, please amend as follows by inserting the following:

RELATED APPLICATIONS

This application is filed under 35 U.S.C. § 1.371 and is the National Stage Application of PCT Application No. PCT/FR2005/050060 filed January 31, 2005, which claims priority to French Application No. 0450199 filed February 3, 2004.

Page 1, line 4, please amend as follows:

~~*Technical field*~~ FIELD OF THE INVENTION

Page 1, line 12, please amend as follows:

~~*State of the art*~~ BACKGROUND OF THE INVENTION

Page 2, lines 14-18, please amend as follows:

The soluble organic elements which are accumulated in the cells accompanying potassium include, quite generally, the organic acids. In fact, the organic acids are the principal chemical species contributing the ~~neutralisation~~ neutralization of the positive charge applied by the potassium accumulated in the cells.

Page 3, lines 1-7, please amend as follows:

In the case of the grape berry, another major organic acid is tartaric acid. The accumulation of tartaric acid is a relatively rare phenomenon in the vegetable kingdom, and in this regard the vine is one of the remarkable exceptions as a species of major agro-economic interest. Tartaric acid is subject to very little degradation in living cells, which means that the quantity ~~synthesised~~ synthesized in a given stage of development remains accumulated for a long time in the cells.

Page 3, lines 20-33, please amend as follows:

~~Technical Problem~~

At the present time it is difficult to influence the size of the storage organs. For example, in order to increase the size of the storage organs to obtain a better yield, ~~fertilisers~~ fertilizers are generally used. Such ~~fertilisers~~ fertilizers are specially selected to meet the nutritional requirements of the plant and to compensate for any nutritional deficiencies of the soil. For example, ~~fertilisers~~ fertilizers supply elements essential for the production of proteins and cellular constituents for the plant.

However, the ~~fertiliser~~ fertilizer may be a product that is harmful to the environment. The addition of ~~fertiliser~~ fertilizer gives rise, in particular, to pollution of the soil and ground water. The discharge of nitrates into the soil poses environmental problems that are difficult to resolve. Moreover, it is necessary to add ~~fertiliser~~ fertilizer regularly because the nutritive elements contained in this ~~fertiliser~~ fertilizer are exhausted during consumption by the plant.

Page 4, between lines 16 and 17, please amend by inserting the following:

SUMMARY OF THE INVENTION

Page 5, lines 1-5, please amend as follows:

One of the objects of the invention is therefore to be able to influence the size and/or composition of organic acids in the storage organs, according to the requirements of the user, without the disadvantages associated, for example, with ~~fertiliser~~ fertilizer additions referred to above.

~~Solutions provided by the invention~~

Page 6, line 1 to page 7, line 5, please amend as follows:

~~*Summary of the invention*~~

The principle object of the invention is therefore a method for obtaining a plant transformed on the basis of a phenotype relative to the size of a storage organ of the plant or the organic acid composition of this organ, ~~characterised~~ characterized in that it comprises the following stage:

- the modification, in the cells of the storage organ or in the tissues supplying the storage organ, the expression of a gene encoding a outward rectifier potassium channel.

The invention therefore also relates to a ~~methods~~ method for selecting a plant on the basis of a phenotype relating to the size of a storage organ of the plant or the organic acid composition of that organ, ~~characterised~~ characterized in that the expression of a gene encoding a outward rectifier potassium channel in the cells of the storage organs or in the tissues supplying the storage organs is measured.

The invention also relates to a cell of a plant, ~~characterised~~ characterized in that it over-expresses a gene encoding a outward rectifier potassium channel whose polypeptidic sequence

has at least a 40% similarity with a polypeptidic sequence deduced from the nucleotidic sequence SEQ ID No. 1.

The invention also relates to a plant, ~~characterised~~ characterized in that it over-expresses a gene encoding a outward rectifier potassium channel whose polypeptidic sequence has at least a 40% similarity with a polypeptidic sequence deduced from the sequence SEQ ID no. 1.

A further object of the invention is the use of a gene encoding a outward rectifier potassium channel to modify in a plant a phenotype relating to the size of at least one storage organ or the organic acid composition of that organ.

A further object of the invention is an antibody, ~~characterised~~ characterized in that it is directed against all or part of a polypeptide resulting from the expression a gene encoding a outward rectifier potassium channel.

The object of the invention is a method for detecting the presence of all or part of a polypeptide resulting from the expression of a gene encoding a outward rectifier potassium channel in a sample containing a mixture of polypeptides, ~~characterised~~ characterized in that it comprises the following stages:

- putting the sample in contact with the antibody previously described, and
- detecting an antigen/antibody complex formed.

And finally, a further object of the invention is a kit for detecting all or part of a polypeptidic sequence resulting from a gene encoding a outward rectifier potassium channel, ~~characterised~~ characterized in that it comprises the antibody previously described.

Page 7, between lines 5 and 6 please amend by inserting the following:

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1: A diagrammatic representation of a potassium channel encoded by the VvSOR gene of *Vitis vinifera;*

FIG. 2: Gel of an electrophoretic migration of products of amplification by quantitative RT-PRC of the RNAm produced by the gene VvSOR in cells or tissues sampled at different points on a transformed plant (TP) or a non-transformed plant (NTP);

FIG. 3: A graphic representation of a quantity of RNAm VvSOR produced by grape berries of *Vitis vinifera* in the course of the growth of such berries. The time reference is the "veraison" stage, which separates the two growth phases C1 and C2.

FIG. 4: A first photograph representing bunches of grapes in the veraison stage, derived from transformed plants; and

FIG. 5: A graphic representation of the accumulated quantities of tartaric and malic acid, on the one hand in the berries harvested at maturity from non-transformed plants (control), and on the other hand in berries harvested at maturity from transformed plants over-expressing the VvSOR gene (over-expresser).

Page 7, lines 13-22, please amend as follows:

As previously mentioned a plant is defined as a vegetable organism comprising a [[steam]] stem and at least one storage organ, the stem being intended temporarily to support this storage organ. Stem is understood to mean [[a]] an axial section of the plant which projects above the soil, grows in the opposite direction to the roots and bears leaves and the storage

organs. Storage organ is defined as any organ capable of being consumed by a living being, such as a fleshy fruit, an oleaginous fruit, seeds, but also vegetables or tubers such as potatoes, for example. The storage organ is temporarily linked to at least one of the ends of the stem. Therefore the sap produced passes from the stem to the storage organs.

Page 8, lines 25-29, please amend as follows:

The foreign gene may be introduced directly into the cell by direct micro-injection of this foreign gene into [[to]] plant embryoids, by ~~infiltrationina~~ infiltration in a vacuum, by electroporation, by direct precipitation by means of polyethylene glycol (PEG) or by gun bombardment of particles covered with the plasmidic DNA of interest.

Page 9, lines 1-11, please amend as follows:

These DNA transfer methods are aimed either at activating an expression of a gene in the cell (or cellular gene), or deactivating this same expression. The cellular gene may be activated either by inserting several copies of the same gene (or foreign gene) in a cellular genome or by placing the encoding sequence of the transgene under the control of a so-called strong promoter (i.e. resulting in a high level of expression), or by inserting a single copy of the gene and a power transcription factor of the gene. The deactivation of the cellular gene may be carried out, for example, by inserting a foreign gene whose RNAm is complementary to the RNAm of the gene of interest. The two RNAm's of cellular and foreign origin are therefore ~~hybridised~~ hybridized and no protein can be produced.

Page 10, lines 13-27, please amend as follows:

Below 40% similarity of polypeptidic sequence, the polypeptidic sequence identified in a given plant species risks not corresponding to a gene encoding a potassium channel similar to the outward rectifier potassium channel encoded by the SEQ ID No.2 gene. Such a sampled sequence may not correspond to a gene likely to be involved in the modification of the phenotype relating to the size or the organic acid composition of storage organs. It is therefore possible to obtain in each of the given species of plants a gene encoding a polypeptidic sequence having at least a 40% similarity with a polypeptidic sequence deduced from the gene of the SEQ ID No1, encoding a outward rectifier potassium channel in *Vitis Vinifera.* For example, a nucleotidic sequence encoding a polypeptidic sequence having at least a 40% [[40$]] similarity with a polypeptidic sequence deduced from the sequence SEQ ID No. 1 in potatoes or in rice may be identified, and such a gene may be used to modify the phenotype relating to the size or organic acid composition of storage organs of these two plants or other plant species.

Page 11, line 30 to page 12, line 27, please amend as follows:

The invention also provides for a marker of a gene comprised in a phenotype relating to a size or organic acid composition of a storage organ. The marker comprises a nucleotidic sequence encoding a polypeptidic sequence having at least a 40% similarity with a polypeptidic sequence deduced from all or part of the nucleic sequence SEQ ID No. 1, or all or part of a nucleotidic sequence complementary to SEQ ID No. 1, or of a nucleotidic sequence SEQ ID No. 2 or SEQ ID No. 3. This percentage similarity takes into account the differences that exist from

one plant species to another, whilst guaranteeing that the gene encodes a potassium channel involved in the size of organic acid composition phenotype of the storage organ, or having a ~~behaviour~~ behavior similar to the potassium channel resulting from the expression of the SEQ ID No. 1 gene.

This marker may be detected by molecular ~~hybridisation~~ hybridization between the nucleotidic sequence of a marker of a given plant and all or part of the sequence SEQ ID 1, the sequence complementary to SEQ ID No.1 or the sequence SEQ ID No. 2 or SEQ ID No. 3.

The nucleotidic sequence of the marker may be in the form of a DNAc, a non-encoding DNA strand or an RNAm. The nucleotidic sequence of the marker may be in any form likely to be detected by molecular ~~hybridisation~~ hybridization with all or part of the sequence SEQ ID No1, the sequence complementary to SEQ ID No. 1, of SEQ ID No. 2 or the sequence SEQ ID No. 3.

The nucleotidic sequences SEQ ID No. 2 and SEQ ID No. 3 form a front primer and a rear primer of the SEQ ID No. 1. These primers enable the presence of the SEQ ID No. 1 to be detected by ~~hybridisation~~ hybridization of the DNAc, the non-encoding DNA or the RNAm deriving from the gene SEQ ID No1. These primers correspond to nucleotidic sequences flanking the gene of the SEQ ID No. 1 sequence.

Page 13, lines 12-20, please amend as follows:

In order to measure the expression of the gene of interest either a measurement of a quantity of RNAm deriving from a transcription of the gene encoding the potassium channel, or a measurement of a quantity of proteins resulting from the expression of this gene is carried out

according to the invention. The measurement of these quantities may be carried out by molecular biology methods well known to the person skilled in the art. For example, the measurement of these quantities may be carried out by a molecular ~~hybridisation~~ hybridization method (Northern Blot), a quantitative reverse-PCR method or by a Western Blot method.

Page 14, lines 1-7, please amend as follows:

The development of the grape berries is ~~characterised~~ characterized by a first growth phase, an intermediate stagnation phase followed by a transition called veraison, then a second growth phase. In particular, the inventors discovered that the quantity of RNAm resulting from the expression of the gene SEQ ID No. 1 was extremely important in the course of the development of the berry, and that the quantity of proteins was very high at the time of development and after the development of the berry.

Page 14, lines 14-20, please amend as follows:

By applying the selection process to cells of a storage organ at a particular time of development, it is advantageously possible to identify the plants having large size storage organs specifically because of the over-expression of the gene, and not due to exposure to sunshine or particularly ~~favourable~~ favorable feeding. Because of this the plants thus identified have a good chance of stably and reproducibly producing new generations of plants that also have this genetic characteristic.

Page 14, lines 28-29, please amend as follows:

Moreover, this method may be a method of verification and may be used to check that the plants have been correctly transformed.

Page 15, lines 4-12, please amend as follows:

For example, the size of storage organ may be quantified by measuring the weight of 100 storage organs for a given wild species. This gives a reference weight of storage organs for a given non-transformed plant. ~~In parallel with~~ Also, the weight of 100 other storage organs of a plant that has been modified according to the invention is measured. These two weights are then compared to deduce from it any transformation of the plant. In one example the inventors discovered that the weight of a storage organ derived from a transformed plant according to the invention was 1.7 times greater than the weight of a storage organ derived from a non-transformed plant.

Page 15, lines 26-33, please amend as follows:

The invention also relates to an antibody directed against all or part of a polypeptidic sequence resulting from the expression of a gene, which polypeptidic sequence has at least a 40% similarity with a polypeptidic sequence deduced from the nucleotidic sequence SEQ ID No. 1. Antibodies, within the meaning of this invention, refer to polyclonal or monoclonal antibodies or fragments (e.g. the fragments F(ab)'2, F(ab) or even fragments comprising a domain of the initial antibody ~~recognising~~ recognizing the polypeptide or the target polypeptide fragment according to the invention.

Page 16, lines 8-30, please amend as follows:

Finally, the invention relates to a kit for detecting all or part of the polypeptide previously described in a sample containing a mixture of polypeptides, ~~characterised~~ characterized in that comprises

- an antibody, previously described, the antibody being detectable by methods of marking the antibody well known to the person skilled in the art.

~~*Figures*~~

~~Figure 1: A diagrammatic representation of a potassium channel encoded by the VvSOR gene of *Vitis vinifera;*~~

~~Figure 2: Gel of an electrophoretic migration of products of amplification by quantitative RT-PRC of the RNAm produced by the gene VvSOR in cells or tissues sampled at different points on a transformed plant (TP) or a non-transformed plant (NTP);~~

~~Figure 3: A graphic representation of a quantity of RNAm VvSOR produced by grape berries of *Vitis vinifera* in the course of the growth of such berries. The time reference is the "veraison" stage, which separates the two growth phases C1 and C2.~~

~~Figure 4: A first photograph representing bunches of grapes in the veraison stage, derived from transformed plants; and~~

~~Figure 5: A graphic representation of the accumulated quantities of tartaric and malic acid, on the one hand in the berries harvested at maturity from non-transformed~~

~~plants (control), and on the other hand in berries harvested at maturity from transformed plants over-expressing the VvSOR gene (over-expresser).~~

Page 18, lines 15-19, please amend as follows:

The RNAm's and the proteins of the samples taken from transformed and non-transformed plants were isolated. Using molecular ~~hybridisation~~ hybridization methods (Northern Blot), quantitative Reverse-PCR and Western Blot), the quantity of RNAm's and proteins derived from the expression of the gene SEQ ID No. 1 was determined.

Page 19, lines 24-31, please amend as follows:

The RNAm's derived from the expression of the SEQ ID No. 1 gene are present in the tail cells of the fruit (S), in the cells of green berries (GB), in the cells of berries at the time of veraison (BV) and in the cells of mature berries (MB). But the RNA's derived from the expression of the gene SEQ ID No. 1 are not present in the roots [[®]] (R), Figure 2. The inventors were therefore able to show that the expression of the gene was observable in the aerial parts of the plant, particularly in the leaves, the young shoots and the berries, but not in the roots.

Page 20, lines 1-9, please amend as follows:

The inventors also estimated the quantity of RNAm produced during the growth of the grape berries of the plant, Figure 3. Figure 3 represents a quantity of RNAm produced during the development of the grape berry as a function of time. In particular, the development of the grape berries is ~~characterised~~ characterized by a first growth stage C1, a stagnation stage followed by a transition stage called veraison, then a second growth stage C2. The inventors observed that the

quantity of RNAm is at its maximum at the time of veraison, indicating that the detection of the transformed plants should preferably be carried out at the time of veraison.

Page 21, line 28 to page 22, line 2, please amend as follows:

After transformation of the cells of grape berries, the cells of grape berries are crushed to extract the RNAm's and proteins at particularly ~~favourable~~ favorable times for detecting an optimum quantity. The quantity of RNAm *VvSOR* produced by the gene of *Vitis vinifera VvSOR* is determined by quantitative Reverse-PCR or molecular ~~hybridisation~~ hybridization (Northern blot), and the quantity of proteins deriving from the translation of this RNAm is determined by immunodetection (Western blot method). Each of these quantities is compared to a quantity of reference RNAm and a quantity of reference proteins corresponding to a quantity of RNAm and proteins normally present in the cells of non-transformed plants respectively.

In the Sequence Listing, please **replace** the Sequence Listing with the following Sequence Listing:

SEQUENCE LISTING

<110> INSTITUT NATIONAL DE LA RECHERCHE AGRONOMIQUE
PRATELLI, REJANE
HOSY, ERIC
LACOMBE, BENOIT
ROMIEU, CHARLES
TORREGROSA, LAURENT
THIBAUD, JEAN-BAPTISTE
SENTENAC, HERVE

<120> Potassium Channels
<130> 3338.100US01
<140> US 10/588,361
<141> 2006-10-24
<150> PCT/FR05/050060

<151> 2005-01-31
<160> 3
<170> PatentIn version 3.5
<210> 1
<211> 2449
<212> DNA
<213> Vitis vinifera
<400> 1

gctaagttgc caagaaaacc agaatagaag agaggccatg aatgatgaat gattggttct 60
ctgtgtctcc catccctact agacagaaca gccacaatgc atcgacaaat gaaatcttcc 120
tgggcacaag acatgaaaga caaaagggaa gaattgaacc tgatggaagg tggtacagaa 180
catggacgaa gttcatattg ttatgggcag tgtactcttc atttttcaca cccatggaat 240
ttggattctt cagaggattg ccagaggacc tggtcttcct ggacattgct ggccagattg 300
cttttcttat cgacattgtg ctccgattct tccttgccta cagggatgcc cacacttacc 360
gcatggtcta caagcgcaca tctattgcac ttaggtatat gaaatctagt tttgtcattg 420
atttaatttg ctgtttgcct tgggatatta tttacaaggc ttgtgggaga aaagaagaag 480
tgaggtacct cttatggatt aggttgatta gggtgtgcaa agtcacagat tttttccaaa 540
acttggaaaa ggacacacgg atcaattata tgtttaccag gattctgaaa cttattgctg 600
ttgaactcta ttgcacgcat acagcagctt gtgtctttta ctatttggct acaacacttc 660
cccaatctga agaggggtac acatggattg gaagtttaaa gctgggggac tacagttact 720
ctcactttag agagattgat atctggaagc ggtacacaac atctttatat tttgcaatca 780
tcacaatggc cactgttggt tacggggata tacatgctgt aaatctgaga gaaatgattt 840
ttgtcatgat ttatgtgtct tttgacatga ttcttggtgc ttacttgatc ggtaacatga 900
cagccttaat agttaaagga tcaaaaactg aaagatttag ggataagatg actgatgtca 960
tcaaatatat gaacagaaac agacttgata gggacgttcg caatcagatc aaaggccacc 1020
tacgcttaca gtatgagagt ggctacactg aagcttctgt tattcaggat cttcctatct 1080
ctattcgagc taagattgcc caaactttat ataagccatt ggttgaaaag gtttctcttt 1140
ttaggggatg ctctttggaa ctcatcaatc aaattgtaat cagagttcat gaggagtttt 1200
ttcttccagg tgaagtgata atggagcagg gaaatgtggt agatcaactt tattttgtct 1260
gtcatggaat gctggaagag ataggcattg gggcagatgg atcagaagaa acagttttgc 1320
ctctgcaacc taacagctcc tttggagaaa tttcaattct ttgcaacatt cctcaaccat 1380
atacagttcg agttcttgaa ttgtgtaggc ttctgcgcct tgataaacaa tctttacaga 1440
taattcttga aatatatttt tatgatgggc ggagaatctt gaacaacctt ctagagggaa 1500
aggaatcaaa tcttcgtgtc aagcaactgg agtcagatat cacatttcat attggtaggc 1560
aagaagctga acttgcttta agggtaaaca gtgcttctta tcatggggat ctatatcagc 1620
taaagagttt gattcgagct ggagctgatc ccaacaagac agattatgat ggaagatcac 1680
ctttgcatct tgcatcagca agaggatttg aagatattgt aactttcctt atacaagaag 1740
gtgtggatgt caatatttca gataattttg gaaacacgcc cttattagaa gccatcaaga 1800
atgcacatga tcgcgttgct tctttgcttg ttaataaagg ggccttgctg aagattgatg 1860
atgctggtgg ttttctgtgt tgcaccattg caaggggtga ttcagatttt cttaaaagga 1920
ttctgtccaa tggcattgat ccaaactcca aagactatga tcacaagacc ccaattcatg 1980
tggctgcctc aggaagggtt atattttatg gcaaagttgc ttttaggaaa gccggggcta 2040
gtgtattctc aaaggacaga tggggaaaca ccccacttga tgaaggttgg aagtgtggaa 2100
acaagaatct aatgaagcta ttggaagatg caaaagtggc tcagctttct gaatttcctg 2160
actgctctag agagatcaca gataaaatgc acccgaggaa atgtactgtg ttccctttcc 2220
acccatggga tccgaaagaa cataaaaggc ctggcatcat gttatgggtc cctcaaacca 2280
ttgaagagct gatcaagact gcaacagagg ggctacaatt ttcaagtgaa tcctgtatat 2340
tatcagagga tggaggtaaa attttggatg tagacatgat aagcgatggt cagaagctat 2400
atttgttatg tgaaacacta gatatttagt gtttcgtctg ctgtaaatc 2449

<210> 2
<211> 28